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US 5045337 A

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(54) **Microemulsions containing functional substances**

(57) A water-in-oil microemulsion which comprises (a) from about 85% to 99.8% by weight of a lipid medium, (b) from 0.1% to 5% by weight of an aqueous medium, and (c) from 0.1% to 10% by weight of a surfactant. The surfactant is a polyglycerol ester of an unsaturated fatty acid having from 12 to 24 carbon atoms and the polyglycerol component of the polyglycerol ester has at least 70% by weight of triglycerols or tetraglycerols. The microemulsions may be used in foodstuffs.

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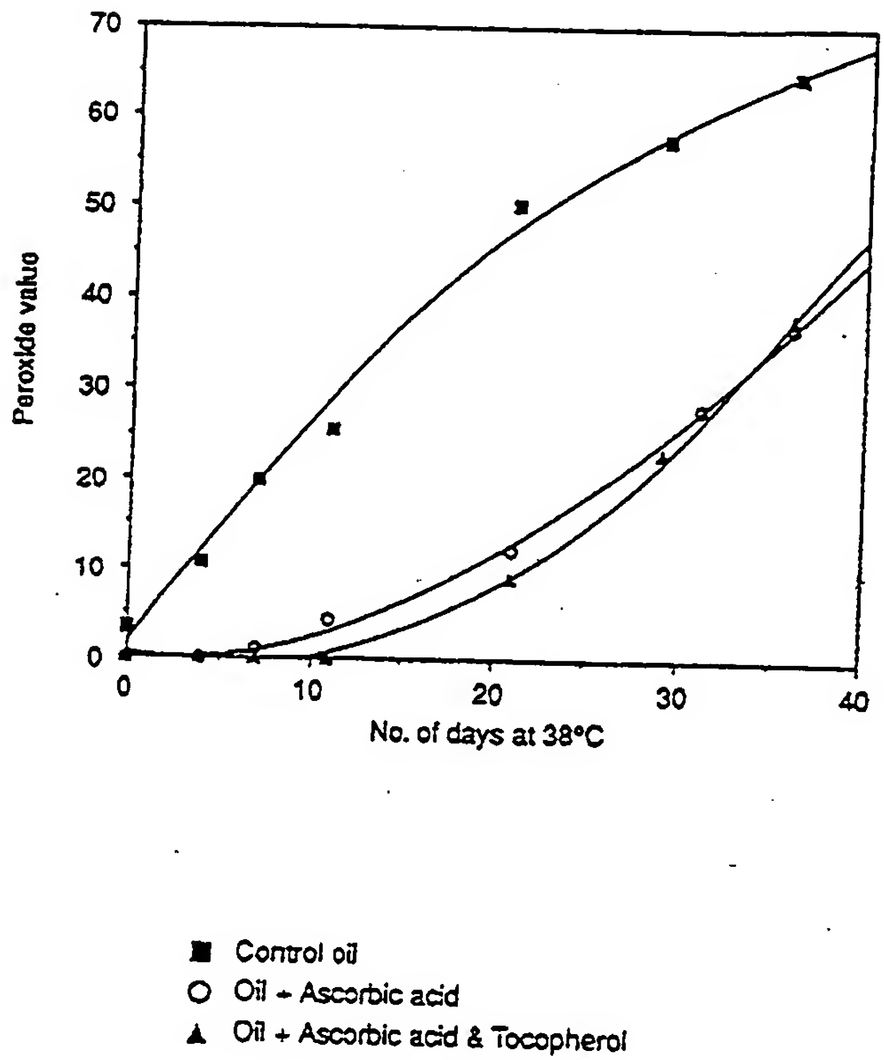
Figure



70
60
50
40
30
20
10
0
Peroxide value
0
10
20
30
40
No. of days at 38°C
Control oil
Oil + Ascorbic acid
Oil + Ascorbic acid & Tocopherol

Microemulsions Containing Functional Substances

This invention relates to water-in-oil microemulsions which may contain a functional substance in the aqueous phase. The microemulsions may be used in foodstuffs.

Many functional substances, such as flavourants, antioxidants, aromas, vitamins, minerals, salts and the like, which are desired to be incorporated into an oil, are water soluble and oil insoluble. Therefore it is not possible to simply mix these water soluble substances into the oil since cloudy dispersions, which with time separate, develop. Also, attempts to incorporate these water soluble substances into the oil using emulsifiers have not in general proved to be successful because milky, unstable emulsions tend to form.

Attempts have been made to formulate microemulsions which contain the functional substances. Microemulsions are optically clear, form spontaneously, and are stable. However these attempts have not proved to be entirely successful because of the difficulty in providing food acceptable surfactants which are able to form microemulsions.

One effective solution to the problem is described in US patent 5045337. Here the aqueous phase, which comprises up to 5% by weight, is formulated into a microemulsion. The surfactant which is used to form the microemulsion is a polyglycerol mono, diester of an unsaturated or branched chain fatty acid having from 12 to 24 carbon atoms, esterified with a polyglycerol consisting of up to 30% diglycerol, 30 to 50% triglycerol, 15 to 50% tetraglycerol, and up to 10% mono and other polyglycerols.

Although the microemulsions described in US patent 5045337 provide an effective solution, it is important to minimize the amount of surfactant which is needed to stabilise a given amount of water. There are at least two reasons for this; one is to reduce costs since the surfactants add to the cost and the other is to keep the amount of substances such as surfactants in food as low as possible and within legal limits. The surfactant used in US patent 5045337 is able to form a microemulsion at a ratio of 12:1 or less of surfactant to water. Example 1 of US patent 5045337 even gets the ratio to as low as 9:1 of surfactant to water.

However, there is a need to further reduce the amount of surfactant required per unit amount of water and to provide other acceptable surfactants.

Accordingly this invention provides a water-in-oil microemulsion which comprises (a) from about 85% to about 99.8% by weight of a lipid medium, (b) from about 0.1% to about 5% by weight of an aqueous medium, and (c) from about 0.1% to about 10% by weight of a surfactant which is a polyglycerol ester of an unsaturated fatty acid having from 12 to 24 carbon atoms, characterized in that the polyglycerol component of the polyglycerol ester comprises at least 70% by weight of triglycerols or tetraglycerols.

Surprisingly, ratios of surfactant to water as low as 5:1 have been shown to produce stable microemulsions. This is a significant improvement over what has been shown in the prior art. That results of this nature may be obtained with a surfactant which has at least 70% triglycerol or tetraglycerol is particularly surprising in view of the teaching in US patent 5045337 which specifically limits these components to an absolute maximum of 50%.

Preferably, the polyglycerol component of the polyglycerol ester comprises less than 10% by weight di-glycerol. Further the polyglycerol component preferably comprises more than 80% by weight tri-glycerol or 75% or more by weight tetraglycerol.

The fatty acid component of the polyglycerol esters preferably comprises more than 50% by weight linoleic acid or oleic acid. More preferably, the fatty acid component comprises 90 % or more by weight linoleic acid. Even more preferably, the fatty acid component comprises substantially pure linoleic acid.

The polyglycerol esters are preferably a mixture of mono-, di- and tri-esters. More preferably the polyglycerol esters contain between 1.5 to 2.5 moles of fatty acid per mole of polyglycerol.

The microemulsion preferably contains less than 2% by weight of the aqueous medium and the weight ratio of aqueous medium to surfactant is 1:10 or less. More preferably, weight ratio of aqueous medium to surfactant is 1:6 or less.

Conveniently, the functional substance in the aqueous medium is a hydrophilic antioxidant; for example ascorbic acid. Incorporating such an antioxidant has the significant advantage of increasing the stability of the lipid medium.

Preferably a lipophilic antioxidant which is soluble in oil is incorporated into the lipid medium. For example, synergistic effects are obtained when ascorbic acid is incorporated into the aqueous medium and α-tocopherol into the lipid medium.

The functional substance may also be an aroma, flavourant, vitamin, salt, mineral, and the like.

In another aspect, this invention provides a water-in-oil microemulsion which comprises (a) from about 85% to about 99.8% by weight of a lipid medium, (b) from about 0.1% to about 5% by weight of an aqueous medium, and (c) from about 0.1% to about 10% by weight of a surfactant which is a polyglycerol ester of an unsaturated fatty acid having from 12 to 24 carbon atoms, characterized in that the surfactant includes mono-, di- and tri-esters and the polyglycerol component of the polyglycerol ester comprises at least 70% by weight of a mixture of triglycerols and tetraglycerols.

The microemulsions may be used as oils which are sold directly to consumers; the functional substance providing the desired effect. For example, the microemulsions may be used as oils which are resistant to oxidation. Also, the microemulsions may be incorporated as the oil or fat component into food products such as confectionery, prepared food dishes, meat products, margarines and low or reduced fat spreads, dressings, dairy products (such as cheese, milk, yogurt, desserts), shortenings, solid fats, and the like.

In this specification, the term "microemulsion" means a thermodynamically stable, clear liquid which forms spontaneously and which contains a lipid phase and an aqueous phase. In order to be clear, the droplets of the dispersed phase have a droplet size less than 150 nm; and preferably less than 100 nm.

Embodiments of the invention are now described, by way of example only, with reference to the drawing which illustrates the oxidative stability of sunflower oil.

The lipid medium is any edible, liquid fat or oil which may be of natural origin (for example vegetable, animal, marine, or mineral) or synthetic origin (for example synthetically prepared triglycerides, polyol polyesters of fatty acids and alcohols, polycarboxylic acid polyesters and the like); or may be such a fat or oil in hydrogenated form. Specific examples of oils and fats include sunflower oil, soybean oil, maize oil, safflower oil, rapeseed oil, cottonseed oil, peanut oil, olive oil, cocoa butter, butter fat, fish oils, and the like, and partially and completely hydrogenated versions thereof. Also conventional low molecular weight synthetic fats, low calorie fats, fat-like sucrose polyesters and the like may be used. Further examples of suitable fats are disclosed in US patent 5045337, the disclosure of which is incorporated by reference.

When the microemulsion is sold directly to consumers (for example in the form of flavoured or stabilised oils), the lipid medium is preferably a solid fat or a clear, liquid oil which would be acceptable to consumers. Oils and fats which are conventionally sold to the consumers would be suitable.

The aqueous medium conveniently contains a desirable functional substance which is water-soluble. Conveniently the functional substance is a flavour, aroma, nutrient, or preservative. Any suitable aromas and flavours, be they natural or synthetic, may be used as desired. Examples are fruit flavours and aromas, herb flavours and aromas, meat or animal fats flavours and the like. The nutrients may be any desired nutrients such as vitamins, trace minerals and the like. Salts and sugars may also be incorporated. Further suitable components are described in US patent 5045337.

Particularly useful however are hydrophilic antioxidants, such as ascorbic acid (which is also a vitamin), which are difficult to incorporate stably into a lipid medium but which would considerably extend the shelf life of the lipid medium. A combination of ascorbic acid in the aqueous medium and α-tocopherol in the lipid medium is particularly useful since these two antioxidants act synergistically to prevent oxidation of the lipid medium when incorporated in the microemulsion.

The surfactant is a mixture of mono- and polyesters of polyglycerols and a fatty acid of 12 to 24 carbon atoms. The polyglycerols are prepared by polymerising glycerol by conventional procedures such as acid or base catalysed dehydration reactions. Usually a mixture of mono- and polyglycerols result. The content of the triglycerols and tetraglycerols is then increased by removing mono-, and di-glycerols using conventional separation techniques. Polyglycerols with enriched tri- or tetra-glycerol contents may also be purchased; for example from Croda Surfactants Ltd, Leek, Staffordshire, UK, Deutsche Solvay Werke, Langhansstrasse 6, 5650 Solingen 11, Germany and Grindsted Products Ltd, Northern Way, Bury St Edmunds, Suffolk, UK.

The fatty acid for use in preparing the surfactant may be any suitable synthetic or naturally occurring fatty acid which has from 12 to 24 carbon atoms. The fatty acid may be saturated or unsaturated. Fatty acids may be derived from vegetable oils such as sunflower oil, maize oil, peanut oil, safflower oil and the like. In these cases, mixtures of different fatty acids result. Preferably, however, substantially pure fatty acids such as linoleic acid, oleic acid, linolenic acids are used. Particularly suitable is linoleic acid as it appears to give superior results.

The polyglycerols may be esterified by reaction with the fatty acids in the presence of a catalyst or by the enzymatic method described in Fregapane *et al*; 1994; Journal of the American Oil Chemists, 71, 87 to 91. The esterification may take place at any or all of the hydroxyl groups and, depending upon the reaction conditions, the number of fatty acids esterified to any polyglycerol may vary. Usually, mono-, di- and tri-esters form the majority of the components. The surfactants described in US patent 5045337 comprise from 1/3 to 2/3 monoesters and the remainder diesters (termed "mono, diesters" in US patent 5045337). No triesters are present; or at least negligible amounts are present. These mono, diesters of this nature also function adequately here. However the surfactant may also include triesters and small amounts of tetraesters; for example the surfactant may have up to 2.5 moles of fatty acid per mole of tetraglycerol.

The microemulsions may be prepared by dissolving the functional component in the aqueous medium; which will usually be water although it may also contain lower alcohols such as ethanol and solvents such as glycerol and

propylene glycol. The lipid medium, the aqueous medium and the surfactant are then mixed together. The order of addition and mixing is usually not relevant. The microemulsion forms spontaneously and quickly with handshaking or vibration. Although not necessary, further mechanical mixing may be carried out.

Specific embodiments are now described by way of example only.

Example 1

The following polyglycerols, which are obtained from Croda Surfactants Ltd and Deutsche Solvay Werke, are used:

Polyglycerol	Composition A	Composition B	Composition C
di-	10 %	-	-
tri-	83 %	2 %	6 %
tetra-	7 %	75 %	91 %
penta-	-	23 %	2 %
hexa-	-	-	2 %

The tetraglycerols of composition B are predominantly linear while those of composition C are predominantly branched.

The polyglycerols of composition B are incubated with lipase and linoleic acid and are esterified using the procedure of Fregapane (*supra*). A sample is dissolved in deuterated methanol and is subjected to ^{1}H NMR spectroscopy using a Jeol EX270 multinuclear spectrophotometer. The 20 protons on the tetraglycerol give an area of 158; indicating 7.9 for each proton. The three methyl protons give an area of 52; indication 17 for each proton. This indicates that the ratio of tetraglycerol to linoleic acid is 1:2.3. A similar result is obtained when the area of the 20 protons on the tetraglycerol is compared to the area of the 4 olefinic protons.

Surfactant prepared from composition A is labelled surfactant A, that from composition B is labelled surfactant B, and that from composition C is labelled surfactant C.

Three samples of 100 mg of commercial sunflower oil are made up. Then 10 mg of one of surfactants A to C are added to each sample. Water is then slowly added to each sample until the system fails to clear; indicating that the system is no longer a microemulsion. The turbidity is measured by spectrophotometry at 600nm. The minimum ratio of surfactant to water which still provides a microemulsion is then calculated.

Surfactant	**Ratio, surfactant to water**
A	7.71:1
B	5.7:1
C	>10:1

Extremely good results are obtained; particularly for surfactant B.

Example 2

The procedure of example 1 is repeated using fatty acids derived from sunflower oil or erucic acid to esterify polyglycerol composition B. The surfactants obtained are labelled D (for the surfactant obtained from sunflower oil) and E (for the surfactant obtained from erucic acid). The effectiveness of each surfactant is then tested as described in example 1.

Surfactant	**Ratio, surfactant to water**
D	8.3:1
E	9:1

Again the results are good.

Example 3

Samples of pure trilinolein are made up. Various quantities of α-tocopherol, up to a maximum of 10 mg/g, are dissolved in the samples. 5 % by weight of surfactant B is then mixed into each sample. Various amounts of

ascorbic acid are then dissolved into water. 0.5 % by weight of the ascorbic acid solution is added to each trilinolein sample to produce oils containing up to 2mg ascorbic acid/g oil. A microemulsion forms in each case. The microemulsions are stored in open vials at 38°C and allowed to oxidise.

The extent of oxidation is determined as the change in concentration of linoleic acid and reported as an oxidation index (100 indicating no oxidation and 0 indicating complete oxidation). The oxidation index is determined by terminating the oxidation by adding BHT in iso-octane, transesterifying the sample by heating at 60°C with methanol and sulphuric acid to form fatty acid methyl esters. The esters are then quantified by capillary gas chromatography.

The number of days taken for the oxidation index to fall below an arbitrary value 85 is then recorded. The data are reported in the following table:

Ascorbic acid	α-Tocopherol concentration			
Concentration	0 mg/g	1 mg/g	2.5 mg/g	10 mg/g
0 mg/g	3.8 days	9 days	17 days	21 days
0.5 mg/g	9 days	60 days	38 days	28 days
2 mg/g	11.5 days	90 days	60 days	34 days

Addition of the ascorbic acid into the aqueous phase increases the stability of the oil. Further increases are obtained upon addition of synergistic amounts of α-Tocopherol into the oil phase.

Example 4

Samples of commercial fish oil and sunflower oil are made up. No or 1 mg/g of α-tocopherol is dissolved in each sample. 5 % by weight of surfactant B is then mixed into each sample. Ascorbic acid is then dissolved into water. 0.5 % by weight of the ascorbic acid solution is added to each oil sample to give a final concentration of 1mg ascorbic acid/g oil. A microemulsion forms in each case. The microemulsions are stored in open vials at 38°C and allowed to oxidise. A control sample containing only oil is also prepared and stored in an open vial at 38°C and allowed to oxidise. The level of oxidation of the oil in each case is measured by determining the peroxide value.

The results obtained for the sunflower oil are given in the figure. The results for the fish oil are similar except that the α-tocopherol has greater effect. This is because sunflower oil naturally contains α-tocopherol.

Again incorporation of the ascorbic acid into the aqueous phase greatly increases the stability of the oil.

Claims

1. A water-in-oil microemulsion which comprises (a) from about 85% to about 99.8% by weight of a lipid medium, (b) from about 0.1% to about 5% by weight of an aqueous medium, and (c) from about 0.1% to about 10% by weight of a surfactant which is a polyglycerol ester of an unsaturated fatty acid having from 12 to 24 carbon atoms, characterised in that the polyglycerol component of the polyglycerol ester comprises at least 70% by weight of triglycerols or tetraglycerols.

2. A microemulsion according to claim 1 in which the polyglycerol component of the polyglycerol ester comprises less than 10% by weight di-glycerol.

3. A microemulsion according to claim 2 in which the polyglycerol component comprises more than 80% by weight tri-glycerol or 75% or more by weight tetraglycerol.

4. A microemulsion according to any one of claims 1 to 3 in which the fatty acid component of the polyglycerol esters comprises more than 50% by weight linoleic acid or oleic acid.

5. A microemulsion according to claim 4 in which the fatty acid component comprises substantially pure linoleic acid.

6. A microemulsion according to any one of claims 1 to 5 in which polyglycerol esters contain between 1.5 to 2.5 moles of fatty acid per mole of polyglycerol.

7. A microemulsion according to any one of claims 1 to 6 which contains less than 2% by weight of the aqueous medium and the weight ratio of aqueous medium to surfactant is 1:10 or less.

8. A microemulsion according to any one of claims 1 to 7 in which the aqueous medium contains a functional substance selected from a water-soluble,

hydrophilic antioxidant, an aroma, a flavourant, a vitamin, a salt, a mineral, or mixtures thereof.

9. A microemulsion according to claim 8 in which the lipid medium contains an oil-soluble, lipophilic antioxidant.

10. A microemulsion according to claim 9 in which the water-soluble, hydrophilic antioxidant is ascorbic acid and the oil-soluble, lipophilic antioxidant is α-tocopherol.

11. A microemulsion substantially as hereinbefore described with reference to the Examples.

The Patent Office

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Application No:	GB 9502745.4	**Examiner:**	Keith Kennett
Claims searched:	1-11	**Date of search:**	17 April 1996

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.O): C5C (CPD); A2B (BKE); B1V (VB)

Int Cl (Ed.6): A23D 7/00

Other: Online: WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
A	US 5045337 (PROCTER) see whole document	1

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
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